Gel Doc[™] XR+ and ChemiDoc[™] XRS+ Imaging Systems with Image Lab[™] Software Instrument Guide

Version 6.0





Gel Doc™ XR+ and
ChemiDoc™ XRS+ Imaging Systems
with Image Lab™ Software
Instrument Guide

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- Image Lab software is based in part on the work of the Qwt project (http://qwt.sf.net).
- Image Lab software is based in part on the work of the Clmg project (http://cimg.sourceforge.net/). See license for details at www.cecill.info/licences/Licence_CeCILL-C_V1-en.html
- 3. Image Lab software is based in part on the work of the Independent JPEG Group (www.ijg.org/).

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Safety and Regulatory Compliance

Important Safety Information

Please read these instructions before attempting to operate the This instrument is suitable for research use only. It must be used, therefore, only by specialized personnel who know the health risks associated with the reagents that are normally used with this instrument.



WARNING! Use of the acrylic screen does not guarantee the user protection from UV radiation. The use of protective eyeglasses, mask, and/or gloves is strongly recommended.

Warranty

The _ is warranted against defects in materials and workmanship for one year. If any defect occurs in the instrument during this warranty period, Bio-Rad Laboratories, Inc. will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

- Defects caused by improper operation
- Repair or modification done by anyone other than Bio-Rad Laboratories, Inc. or the company's authorized agent
- Use of spare parts supplied by anyone other than Bio-Rad Laboratories,
- Damage caused by accident or misuse
- Damage caused by disaster

Corrosion caused by improper solvents or samples

General Precautions

- Read the user guide carefully.
- The instrument must be used only for the intended purpose of gel documentation in research laboratories.
- The instrument must be connected to a grounded power source line and protected by a circuit breaker.
- Do not pour liquids directly on or inside the instrument.
- Switch off all lights on the instrument immediately after use.
- Clean the transilluminator sample area after use.

Regulatory Notices

The Gel Doc XR+ or ChemiDoc XRS+ imaging system is designed and certified to meet EN 61010, the internationally accepted electrical safety standard, EMC regulations, and TUV requirements. Certified products are safe to use when operated in accordance with this user guide. Do not modify or alter this instrument in any way. Modification or alteration of this instrument will

- Void the manufacturer's warranty
- Void the regulatory certifications
- Create a potential safety hazard



Alert Icons

Alert icons in this guide call attention to caution and warning paragraphs. Each icon indicates the type of hazard addressed.

Table 1. How alert icons are used in this guide

Table 1. How	alert icons are used in this guide
Icon	Explanation
A	General
	Indicates a potential hazard requiring special attention. This icon is used when the hazard or condition is of a general nature.
^	Electrical hazard
4	Indicates a potential hazard requiring special attention when you are working with electricity or electrical equipment.
A	Extreme heat and flammable materials
<u> </u>	Indicates a potential hazard requiring special attention when you are working with extreme heat and flammable materials.
^	Radiation hazard
((0 1)	Indicates a potential hazard requiring special attention when you are working with UV radiation.

Cautions

A caution in this guide alerts you to take or avoid a specific action that could result in loss of data or damage to the instrument. A caution can also indicate that, if the precaution against a potential hazard is not taken, minor or moderate injury might occur.

Example



Caution: With the exception of cleaning or replacing light bulbs, refer all servicing to qualified Bio-Rad personnel or their agents.

Warnings

A warning in this guide precedes an action that, if not followed correctly, could cause serious injury or death to the operator, serious or total loss of data, or serious damage to the instrument.

Example



WARNING! Use of the acrylic screen does not guarantee the user protection from UV radiation. The use of protective eyeglasses, mask, and/or gloves is strongly recommended.

Instrument Safety Warnings

Before you operate the instrument, carefully read the contents of Table 2.

Table 2. Safety cautions and warnings for the instrument

Icon Meaning



Caution: With the exception of cleaning or replacing light bulbs, refer all servicing to qualified Bio-Rad personnel or their agents. If you experience technical difficulties with the instrument, contact Bio-Rad to schedule service. The instrument should not be modified or altered in any way. Alteration voids the manufacturer's warranty and might create a potential safety hazard for the user.



Caution: If the case interlock is defeated, there is a possibility of UV-B radiation hazard due to UV-B light exposure. Exercise caution when servicing the instrument.



Caution: Disconnect the AC power cord before removing the instrument cover.



Warning: This instrument must be connected to an appropriate AC voltage outlet that is properly grounded.

Notice

The Gel Doc XR+ or ChemiDoc XRS+ imager is intended for laboratory use only. This device is meant for use by specialized personnel who know the health risks associated with reagents normally used in electrophoresis. Bio-Rad Laboratories, Inc. is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or for instrument modifications not performed by Bio-Rad Laboratories, Inc. or an authorized agent.

Power Safety Information

Voltage Setting Information

The universal hood for the Gel Doc XR+ or ChemiDoc XRS+ imager has a power supply that automatically chooses the correct voltage for your country or region.

Fuses

The universal hood for the Gel Doc XR+ or ChemiDoc XRS+ imager has two userserviceable fuses, F1 and F2, which are located on the bottom rear panel and are a part of the power entry module. See Fuse Replacement on page 48 for more information.

Safety and Regulatory Compliance

Introduction

Gel Doc™ XR+ and ChemiDoc™ XRS+ gel imaging systems contain a charge coupled device camera (CCD) to capture images in real time. Image Lab™ software facilitates accurate positioning of the sample and generates optimized image data.

Each imager uses a lighttight enclosure (the universal hood) containing built-in UV and white light illumination. Both systems feature dynamic flat fielding technology for image uniformity and quantitation. Image Lab software controls image capture and optimization for the selected applications, analyzes results, and produces a report in a format you choose.

This instrument guide describes the Gel Doc XR+ and ChemDoc XRS+ imagers and includes general procedures for acquiring images. See the Image Lab Software User Guide for more detailed information about working with Image Lab software.

Gel Doc XR+ Imaging System

Gel Doc XR+ imaging system is a high-resolution gel documentation system for quantitation of gels and blots. Position the sample inside the imager and follow the onscreen steps to run a protocol with one click. You can create custom applications for a protocol or create a new protocol using many options in Image Lab software.

Features include

- Detection of dynamic ranges that cover >3 orders of magnitude
- Dynamic flat fielding specific to fluorescent and colorimetric applications

- Smart, application-based protocol setup using Image Lab software, which presents appropriate filter and illumination sources for each application
- Ability to upgrade to the ChemiDoc XRS+ system

ChemiDoc XRS+ Imaging System

In addition to the gel applications you can use with the Gel Doc XR+ imaging system, the ChemiDoc XRS+ imaging system offers sensitive chemiluminescent detection. The imager includes a supersensitive CCD camera that is deeply cooled for the long imaging times required for faint sample detection and image data quantitation.

Features include

- Suitability for imaging applications that require excellent sensitivity (chemiluminescent western blots)
- Smart, application-based protocol setup using Image Lab software, which presents appropriate filter and illumination sources for each application
- Dynamic range >4 orders of magnitude
- Flexibility to image chemiluminescent, fluorescent, and colorimetric samples with dynamic flat fielding specific to each application

System Components

CCD Camera and Lenses

The CCD camera, equipped with a motorized zoom lens (MZL), sits on top of a lighttight enclosure (the universal hood). After you perform initial calibration, a patented* algorithm controls the MZL to focus the lens automatically. Using Image Lab, you can control the MZL remotely to adjust zoom, focus, and iris.

*U.S. patent 8913127

For camera specifications of each system, see the following:

- Gel Doc XR+ Technical Specifications on page 18
- ChemiDoc XRS+ Technical Specifications on page 22

Note: A factory installed +1 diopter lens makes the entire sample stage visible. This lens should always remain on the MZL assembly.

Universal Hood

The universal hood captures fluorescent and chemiluminescent images without using a photographic darkroom. The enclosure has built-in white light epiillumination and UV transillumination. For easy sample loading, the UV transilluminator is located in the universal hood drawer and can be accessed from the front of the imager. When not imaging, the lights in the darkroom enclosure turn off automatically.

The universal hood has touchpad buttons to perform various functions, but you do not have to control the lens and lights manually. Instead, you can use Image Lab software to control all functions remotely. Running a protocol overrides touchpad input.

Image Lab Software

Each system includes Image Lab software. In addition to controlling the imaging systems and acquiring and optimizing images, you can do the following in Image Lab:

- Annotate and document images
- Analyze molecular weights when imaging protein and nucleic acid gels
- Determine quantitation and purity of samples

You can print all or a subset of data in a report. You can also export data to other software, such as Microsoft Office[®], for further analysis or presentation options. See the Image Lab Software User Guide for detailed instructions.

Emission Filters

The universal hood can hold two different emission filters for fluorescent applications. No filter is required to image chemiluminescent samples.

A standard filter (catalog #1708081) is used for colorimetric (white light) applications and is included in the installation kit.

Optional Accessories

Bio-Rad Laboratories, Inc. offers a selection of optional filters and illumination sources. See Appendix C, Accessories, for ordering information and a complete listing of accessory filters, UV light sources, optional parts, and replacement parts.

Printer

For your convenience, Bio-Rad offers an optional USB printer for use with the Gel Doc XR+ and ChemiDoc XRS+ systems: the Mitsubishi thermal printer (catalog #1708089). For more information about the Mitsubishi thermal printer, see the Image Lab Software User Guide.

Conversion Screens

White Light Conversion Screen

The white light conversion screen (catalog #1708001) is a phosphor screen that produces white light transillumination when placed on top of the UV transilluminator.

XcitaBlue Conversion Screen

The optional XcitaBlue™ screen (catalog #1708182) is a UV to blue light conversion screen, which enables you to visualize DNA samples while protecting them from UV damage.

Gel Doc XR+ Imager

Refer to the Gel Doc™ XR+ Installation Guide in the installation kit for information about installing and calibrating the imaging system. To recalibrate the imager when you acquire new accessories, refer to Chapter 5, System Calibration.

Gel Doc XR+ Imager Workflow

Following are the basic steps for acquiring, analyzing, and archiving an image using the Gel Doc XR+ imager and Image Lab™ software:

- Select a protocol or create a new one.
- 2. Position the sample you want to image.
- 3. Run the protocol.
- 4. View the results.
- 5. Optimize the analysis.
- 6. Generate a report.
- 7. Save or export the results.

Gel Doc XR+ Technical Specifications

Applications	
Chemiluminescence	No
Fluorescence*	Yes
Colorimetry	Yes
Gel documentation	Yes
Hardware Specifications	
Maximum sample size	Length: 28 cmWidth: 36 cm
Maximum image area	Length: 19.4 cmWidth: 26 cm
Excitation source	 Trans-UV and epi-white are standard (302 nm included, with 365 nm available as an option). Optional trans white, self-powered or conversion screen. Optional XcitaBlue™ UV/blue conversion screen.
Illumination control	3 modes: Trans-UV Trans white Epi-white
Detector	CCD
Pixel size	4.65 x 4.65 (H x V in microns)
Cooling system	Not applicable
Camera cooling temperature	Not available
Filter holder	3 positions:2 for emission filters1 for using no filter
Emission filters	1 included (standard)3 optional

Dynamic range	>3.0 orders of magnitude
Pixel density (gray levels)	4,096
Dynamic flat fielding	Application-specific, for all applications
Instrument size	Length: 36 cm
	Width: 60 cm
	■ Height: 96 cm
Instrument weight	32 kg
Operating Ranges	
Operating voltage	110/115/230 V AC nominal
Operating temperature	10–28°C (21°C recommended)
Operating humidity	<70% noncondensing
Automation Capabilities	
Workflow automated selection	Application driven, user selected or recalled by a protocol
Workflow automated execution	Controlled by a protocol via application-specific setup for image area, illumination source, filter, analysis, and reporting
Workflow reproducibility	100% repeatability via recallable protocols; from image capture to quantitative analysis and reports
Autofocus	Precalibrated focus for any zoom setting
Image flat fielding	Dynamic; precalibrated and optimized per application
Autoexposure	2 user-defined modes (intense or faint bands)

^{*} When acquiring images from SYBR® Safe DNA applications, Bio-Rad highly recommends that you use the optional XcitaBlue Conversion Screen kit (catalog #1708182), which enables you to visualize DNA samples and protects them against UV damage.

ChemiDoc XRS+ Imager

Refer to the ChemiDoc™ XRS+ Installation Guide in the installation kit for information about assembling and calibrating the imaging system. To recalibrate the imager when you acquire new accessories, refer to Chapter 5, System Calibration.

In addition to the applications that can be used with the Gel Doc XR+, the ChemiDoc XRS+ can acquire images from chemiluminescent blots.

ChemiDoc XRS+ Imager Workflow

Following are the basic steps for acquiring, analyzing, and archiving an image using the ChemiDoc XRS+ system and Image Lab software:

- Select a protocol or create a new one.
- 2. Position the sample to be imaged.
- 3. Run the protocol.
- 4. View the displayed results.
- 5. Optimize the analysis.
- 6. Generate a report.
- Save or export the results.

When running chemiluminescent blots, you can use signal accumulation mode (SAM), which takes a series of exposures in a set period of time. You can then choose the image with the optimal signal. Refer to Chapter 3, Acquiring Images for more information.

ChemiDoc XRS+ Technical Specifications

Applications	
Chemiluminescence	Yes
Fluorescence*	Yes
Colorimetry	Yes
Gel documentation	Yes
Hardware Specifications	
Maximum sample size	Length: 28 cmWidth: 36 cm
Maximum image area	Length: 26 cmWidth: 35 cm
Excitation source	 Trans-UV and epi-white are standard (302 nm included, with 365 nm available as an option)
	Optional trans white, self-powered or conversion screen
	■ Optional XcitaBlue™ UV/blue conversion screen
Illumination control	5 modes:
	Trans-UV (standard)Epi-white (standard)
	No illumination for chemiluminescence (standard)
	Trans white (optional)
	XcitaBlue (optional)
Detector	Supercooled CCD
Pixel size	6.45 x 6.45 (H x V in microns)
Cooling system	Peltier cooled
Camera cooling temperature	-30°C controlled
Filter selector	3 positions:
	■ 2 for filters
	1 without filter (for chemiluminescence)

Emission filters	1 included (standard)
	■ 3 optional
Dynamic range	>4.0 orders of magnitude
Pixel density (gray levels)	65,535
Dynamic flat fielding	Application-specific, for all applications
Instrument size	■ Length: 36 cm
	■ Width: 60 cm
	■ Height: 96 cm
Instrument weight	32 kg
Operating Ranges	
Operating voltage	110/115/230 V AC nominal
Operating temperature	10–28°C (21°C recommended)
Operating humidity	<70% noncondensing
Automation Capabilities	
Workflow automated selection	Application driven, user selected or recalled by a protocol
Workflow automated execution	Controlled by a protocol via application-specific setup for image area, illumination source, filter, analysis, and reporting
Workflow reproducibility	100% repeatability via recallable protocols; from image capture to quantitative analysis and reports
Autofocus	Precalibrated focus for any zoom setting or sample height
Image flat fielding	Dynamic; precalibrated and optimized per application
Autoexposure	2 user-defined modes (intense or faint bands)

^{*} When acquiring images from SYBR® Safe DNA applications, Bio-Rad highly recommends that you use the optional XcitaBlue Conversion Screen kit (catalog #1708182), which enables you to visualize DNA samples and protects them against UV damage.

Acquiring Images

Image Lab™ software runs configurable application-based, protocols for sample imaging. In a single acquisition, a protocol runs a combination of settings for acquiring an image, analyzing it, and creating a customized report.

Protocols can be retrieved, revised, and reused.

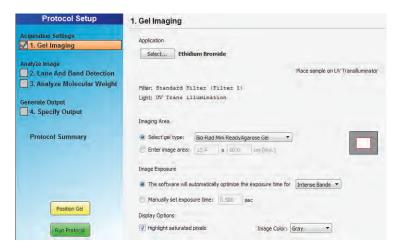
Images you acquire at high resolution result in large files. For this reason, Bio-Rad recommends archiving images by exporting the files to network file server or to removable storage media.

For more detailed information about protocols, see the Image Lab Software User Guide.

Creating Protocols

Creating a protocol consists of configuring acquisition, analysis, and output settings. Analysis and output settings are optional.

To create a protocol, you configure these settings on the Protocol Setup screens.



The title bar above the Protocol Setup screen displays the protocol name and imager type. Main steps appear as headings in the left pane of each screen. Numbered steps appear under these headings.

Analysis settings consist of the following:

- Detecting lanes and bands
- Analyzing molecular weight

Output settings consist of the following:

- Printing the image
- Printing the report
- Displaying the report

To choose options for a protocol step, select the checkbox to the left of the numbered step. To disable a numbered step, clear its checkbox.



Options for the selected step appear on the right side of the screen.

To determine the optimum imaging time for a chemiluminescent sample, select the signal accumulation mode (SAM) option.

Using Signal Accumulation Mode (SAM)

(ChemiDoc[™] XRS+ only) When you run a chemiluminescence application, you can choose signal accumulation mode (SAM) under Image Exposure on the Gel Imaging protocol setup screen.

SAM simplifies obtaining an optimal image from a chemiluminescent sample. This sample type often requires long integration times to obtain an image that represents the best range of signal. SAM acquires a number of images with different imaging times so you can compare the images and then select the optimum imaging time.

SAM presents a series of cumulative images with progressively greater signal in each image. To run SAM you must estimate the shortest and longest times expected to generate an image with the appropriate signal intensity. You then decide how many total images to acquire in this period of time.

For example, if the minimum expected time to image the sample is 1 minute and the maximum is 5 minutes, you enter these values (in seconds) in the Setup dialog box. The value you enter in the Total number of images box defines the number of images SAM captures in the defined interval.

4 | Acquiring Images

In this example, images are acquired at 1 minute intervals, starting at 1 minute and ending at 5 minutes. The second 1-minute image is added to the first 1-minute image, and the final image is the result of integrating these two images. The third 1-minute image is added to the previous image, and so on, until the last image is presented.

Although SAM is useful for determining the optimum imaging time for a chemiluminescent sample, it results in data that are not as accurate as data from a single image. Signal that is near the intensity of background noise becomes increasingly masked as the number of cumulative images grows. To identify extremely faint signals in an image, reacquire it as a single image, using the time the SAM tool found to be appropriate.

Note: For more information about acquiring and saving SAM images, see Running a Protocol with SAM in this chapter.

Accessing Protocol Setup Screens

To access the default Protocol Setup screen

1. Verify that the name of your imager appears on the status bar.

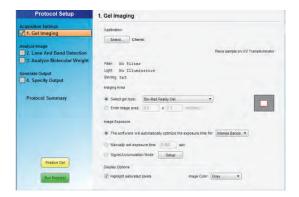


The green check mark indicates that the imager and the computer running Image Lab software are communicating with each other.

2. Click New Protocol on the toolbar.



The Protocol Setup screen appears. Gel Imaging is selected in the left pane.



Gel imaging options appear on the right side of the Protocol Setup screen. The protocol name appears in the title bar. You can change this name when you save the protocol.

Choosing an Application

The term *application* refers to sample type. The sample types consist of the following options:

- Nucleic acid gel, protein gel, or blot
- Detection reagent dye or stain

In the Gel Imaging Protocol Setup screen, you can choose from a list of predefined applications for common sample types. Each sample type is predefined with optimal acquisition settings.

To use a dye or stain not listed in the Application dropdown menus, you can choose the application type Custom and create a custom application. For more information, see Setting Up a Custom Application on page 37.

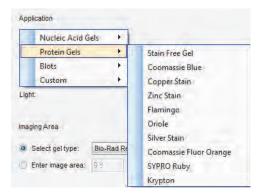
Configuring a Protocol

On the Protocol Setup screen toolbar, click New Protocol.

The Protocol Setup screen appears with Gel Imaging selected. Acquisition options appear in the right pane.

To configure acquisition settings

- 1. Under Application, click Select an application type.
- 2. In the dropdown menu that appears, choose a predefined application.

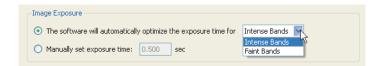


Note: When you select Stain Free Gel, you can also select the gel activation time. See Appendix D, Using Bio-Rad Stain-Free Technology, for more information.

 Under Imaging Area, select the appropriate gel type or enter image area dimensions. The red box to the right represents the imaging area for the selected gel. The gray rectangle represents the imager sample stage.

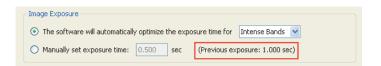


Under Image Exposure, select one of the following:



- **Auto Exposure** estimates an optimal exposure time and ensures the best use of the dynamic range.
 - Intense Bands optimizes exposure for all bands.
 - Faint Bands uses a longer exposure time, making faint bands more visible; more prominent bands might be overexposed.

After imaging a gel optimized for automatic exposure, the exposure time used appears on screen. You can use it as a reference to set a manual exposure time.



- *Manual Exposure* overrides the automatic option. Exposure time can range from 0.001–99 seconds (Gel Doc™ XR) and from 0.001–7,200 seconds (ChemiDoc XRS+). You can view the image exposure time in the Image Info dialog box, which you can access in the Display Toolbox above the on-screen image.
- 3. (ChemiDoc XRS+ only) Signal Accumulation Mode when you run a chemiluminescence application, you can also select SAM. To do so, select it under Image Exposure.



Display Options — two settings enable you to fine-tune image appearance:

4 | Acquiring Images

- Highlight saturated pixels display saturated pixels in red, which
 indicates how much of the gel image is saturated. You can change this
 option later: select View > Image Transform
- 5. Save the protocol and run it or go to the analysis and output settings.

Configuring Analysis Settings

To analyze a gel or blot automatically, configure analysis settings in the following protocol setup tasks:

- Detect Lanes and Bands
- Analyze Molecular Weight

For information about these settings, see the chapter, Protocols, in the Image Lab Software User Guide.

Configuring Output Settings

When you display a report, a scrollable report screen appears in which you can view the image, acquisition settings, and analysis data. You can view or print a single image or report.

Note: You cannot print a report on a thermal printer.

To specify protocol output

1. Select Specify Output in the left pane of the Protocol Setup screen.

Output options appear in the right pane.



2. Choose one of the following:

- Automatically print the image
- Automatically print a report
- Display the report

Image Lab prints to the default printer unless you select another printer.

See the chapter, Generating Reports, in the Image Lab Software User Guide for information about customizing reports.

Positioning the Gel

After configuring protocol setup steps, position the gel on the imaging stage and adjust the camera zoom level in Image Lab.

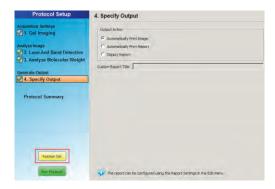
With the Bio-Rad gel alignment template kit you can center four sizes of standard agarose gels and ensure the consistent placement of each gel. See Appendix C, Accessories for more information.

Tip: To review protocol settings, click Protocol Summary in the left pane.

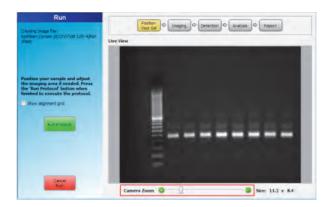
4 | Acquiring Images

To position a gel

- 1. Place a gel on the imaging stage and center it.
- 2. In Image Lab, click Position Gel in the Protocol Setup screen.

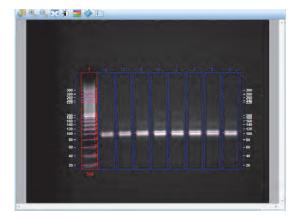


3. The Run screen appears.



- 4. Adjust the camera zoom level with the slider below the image.
- 5. Click Run Protocol.

After the protocol runs, the acquired image appears.



For more information about running a protocol, see the chapter, Protocols, in the Image Lab Software User Guide.

Running a Protocol with SAM

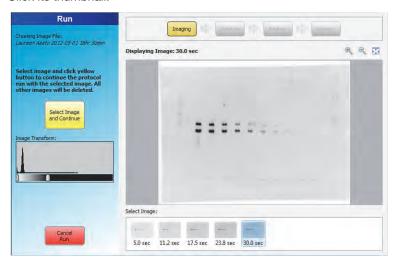
Note: Only the ChemiDoc XRS+ imaging system works with Signal Accumulation Mode (SAM). SAM can be used only with chemi applications.

You can interrupt the acquisition of images for a SAM-enabled protocol at any time. To do so, click Stop Acquire and Continue with Selected. The acquisition process stops and then continues the protocol with the image you select. Images already acquired are discarded.

4 | Acquiring Images

To view a SAM image in the workspace

Click its thumbnail.



To save the image you want to analyze

- Review the images and identify the image you want to use in your analysis.
 - Important: Save the image you want to keep before you continue running the protocol. When you click Select Image and Continue, the selected image is saved and all unsaved images are deleted.
- 2. Select the image you want and click Select Image and Continue.
 - Image Lab continues to the next step in the protocol using the image you selected.

Saving SAM Images

To save a SAM image

- 1. Right-click its thumbnail and click Save.
- In the Save File dialog box, accept the default file name or enter another name. Click Save.

To save all SAM images

- Right-click a SAM image and select Save All.
- 2. In the Select Directory dialog box, enter a folder name and click Choose.

The images are saved in the specified folder. The name of the file includes the user name, timestamp, and exposure time. For example:

John Doe 2015-05-01 15 hr 44 min Exposure 5.0sec.

Setting Up a Custom Application

When you create a protocol, Image Lab displays a list of application types and predefined applications for acquiring images. To use a dye or stain not listed in the Application dropdown menus, you can choose the application type Custom and create a custom application. Also choose Custom to do the following:

- Choose a previously saved custom application
- Edit or rename a custom application

Note: If you are not sure how to configure a custom application for a dye or stain, contact Bio-Rad Technical Support.

To create a custom application

- On the Protocol Setup screen, select 1. Gel Imaging.
- 2. Under Application, click Select and choose Custom on the menu that appears.

The Manage Custom Applications dialog box appears.

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3. Click New.

The Create Custom Application dialog box appears.



- 4. Enter a unique application name.
- 5. Under Application Settings, select a light source, filter, and image color.

Viewing the image with a different color scheme can make all of its elements more visible.

(ChemiDoc XRS+ only) Select a binning setting.

A higher binning setting combines pixels to increase the amount of signal without increasing noise. A higher setting provides optimal sensitivity for low-light applications such as chemiluminescence, but it reduces image resolution.

Editing a Protocol

You can open a protocol, change its settings, and save it with another name. You can also disable a step in the saved protocol. When you edit a default protocol and save it with a new name, the default protocol is unaffected.

See the chapter, Protocols, in the Image Lab Software User Guide for more information.

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5 System Calibration

When the imager is installed, it is calibrated using a calibration wizard. For detailed instructions, see the installation guide in the imager installation kit.

The Image Lab™ software instrument calibration wizard provides several options required to automate the system and prevent focus problems. Each of these calibrations affects the system as follows:

- Focus Calibration allows automated focus settings at any zoom point, using a software algorithm. This ensures that the focus remains correct whether you view an entire sample or an area of interest.
- Focus Calibration with Height Offset —takes the tallest of the available conversion screens into account and extrapolates values for the others so that focus remains optimal for the screen in use. Checking the Illumination options on the Instrument Setup screen instructs Image Lab to generate the focus calibration offset. Focus calibration offset does not change flat field calibrations.
- Dark Reference Image determines and corrects any background signal present in the imager. (Gel Doc™ XR+ imaging system only)
- UV Flat Field Calibration —generates the flat field correction profiles required for the UV light source. The orange flat field generates a flat field profile for UV. Because of this calibration, the images have more accurate quantity reporting and backgrounds of even intensity.
- **Lens Flat Field Calibration** corrects for the intensity roll-off inherent in any lens.

- White Conversion Screen Calibration —generates a flat field correction profile required for the white light conversion screen.
- Bias Value Correction corrects bias for a no-light baseline camera readout. (ChemiDoc™ XRS+ system only)

Recalibrating the Imager

When you add light sources or filters to the Gel Doc XR+ or ChemiDoc XRS+ imager, you must recalibrate the imager.

To recalibrate the imager

- In Image Lab, click Edit > Instrument Setup.
- 2. The Instrument Setup dialog box appears.



(Optional) To add a new light conversion screen, select the appropriate box in the Illumination Options field.

For more information, see Appendix C, Accessories.

4. For any other changes to the optical pathway, perform a flat field calibration. To do so, click Reset in the Instrument Calibration > Flat Field group and follow the on-screen instructions.

Note: When you use the imager to conduct quantitation with Bio-Rad stain-free gel technology, Bio-Rad recommends performing the flat field calibration with the orange fluorescence reference plate (catalog # 1708008) after installing Image Lab 6.0. For instructions, see Orange Fluorescence Reference Plate on page 58.

- 5. Wait for the software to prompt you to recalibrate the new illumination sources.
- 6. Click OK.

Maintenance

UV Transilluminator Lamp and Starter Replacement

Important: Keep the UV filter surface clean from the chemical agents used as gel dyes. Use protective gloves when touching the UV transilluminator cover.

Depending on usage, the UV bulbs and starters can last for many years. Replace bulbs when you notice them flickering. If a bulb does not turn on when it is new or moved, replace the bulb starter and test the bulb again.

Two types of bulbs are available. The catalog numbers are listed in Appendix C, Accessories. The standard bulb is 302 nm. Optionally, the 365 nm bulb is used to minimize denaturing of DNA.

To replace the lamps

- 1. Turn off the power.
- 2. Disconnect the power cord from the universal hood.
- 3. Remove the four screws located on the left/right sides of the transilluminator cover.
- 4. To remove the cover with the UV glass, slide it forward and then lift it up.
 - Note: Do not put the UV cover directly on the bench. Wear gloves when touching the lamps.
- 5. Place the cover on a nonabrasive surface so that the glass does not get scratched or damaged.

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Rotate the lamp until it becomes loose and the pins come to a vertical position.



- Remove the lamp.
- 8. Install the new lamp by rotating it so that the pins are horizontal and the lamp is tight.
- Remove the starter by rotating it counterclockwise and then pulling it out.



- 10. Insert the new starter into the holder and rotate it clockwise.
- 11. Reassemble the cover and retighten the screws on both sides.

Epi-Illumination Lamp Replacement

The lamps are located behind two cover panels on the left and right sides of the universal hood's interior.



To replace the lamps

- 1. Turn system power off and remove the power cord.
- 2. Open the enclosure door.
- 3. Locate the epi-white light housing inside the hood and the screw on the center of the housing cover.
- 4. Use a socket wrench to remove the screw.
- 5. Pull the cover to remove it from the housing.
 - The lamp is now visible.
- 6. To remove the lamp, hold it at the plastic receptacle and pull it from the receptacle.
- 7. Insert the new lamp into the lamp receptacle and push until it is firmly seated in the receptacle.
- 8. Reassemble the epi-white light cover.

Fuse Replacement



Caution: Always unplug the instrument before changing or checking the fuses.

This unit is protected by two fuses (5 x 20 mm, 2A Slo-Blo). The fuses are located in the left side rear of the universal hood, in a fuse holder housed in the power entry module.



To replace the fuses

- Unplug the main power cable from the power outlet.
- 2. Use a screwdriver as a lever on the fuse holder to extract the fuse.
- 3. Remove the blown fuses and replace them with two new fuses (catalog #9008935).
- 4. Slide the fuse holder into the power entry module until it snaps into place.

Troubleshooting В

The following table lists potential problems and suggested solutions.

Problem	Possible Cause	Solution		
Camera does not respond/camera not found	Power to the camera may be turned off.	Turn on the power to the camera.		
	The camera cables might not be seated properly.	 Make sure that all cables are connected as shown in the Installation Guide. 		
	The software driver for the camera is missing.	■ If the camera driver is not present, reload the camera driver from the Image Lab™ software CD.		
	 Computer power-saving modes might be interfering with the camera driver. 	 Disable the power-saving modes on the computer. 		
	The cables might be defective.	Replace the cables.		
	The camera might be defective.	Replace the camera.		
Horizontal stripes in image when using the UV mode	The emission filter might not be positioned properly.	 Cycle power to the Universal Hood III so that the filter wheel is positioned properly. 		
Image is not visible on the monitor	The monitor settings are incorrect.	 See your computer manual for the proper settings. 		
	The lens cap is attached.	Remove the lens cap.		

B | Troubleshooting

Possible Cause	Solution	
The monitor settings are wrong.	See your monitor manual for the appropriate settings.	
The printer settings are wrong.	See your printer manual for the appropriate settings.	
The lens body is not seated properly against the gasket on the hood's adapter plate.	Loosen the thumbscrew and seat the lens properly against the gasket on the hood's adapter plate.	
 Focus is not calibrated for samples using this light source. 	Select Edit > Instrument Setup to recalibrate the focus for use with this accessory.	
The camera lens is not seated properly on the lens mounting plate.	Re-seat the camera on the lens mounting plate.	
	 The monitor settings are wrong. The printer settings are wrong. The lens body is not seated properly against the gasket on the hood's adapter plate. Focus is not calibrated for samples using this light source. The camera lens is not seated properly on the 	

C Accessories

Calibrating Accessories

When you install accessories during initial installation of the imaging system, you must run the one-time Instrument Calibration wizard to calibrate the system. Refer to the installation guide for instructions.

You must also recalibrate the imager before using new conversion screens, light sources, or filters. See Chapter 5, System Calibration for more information about calibrating new accessories.

Installing Optional Accessories

UV/White Light Conversion Screen

This optional white light conversion screen (catalog #1708289) converts the UV light generated in the universal hood to white light. The imager must be calibrated to use the white light conversion screen.

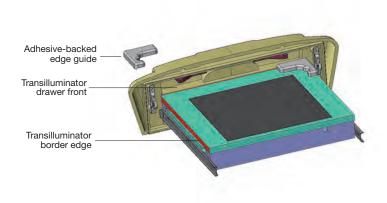


The UV/White Light conversion screen is held in place by adhesive-backed edge guides. After the edge guides are installed, the conversion screen remains centered and does not slide, even when you close the drawer rapidly.

To install the UV/White Light conversion screen

Important: Do not remove the adhesive paper tape from the edge guides until step 2. You cannot reposition the adhesive surfaces of the edge guides once they are set in place.

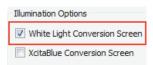
 Holding the screen right side up with the paper taped edge guides underneath, practice positioning the edge guides so that they touch the inside of the transilluminator drawer front and fit over the edge of the metal transilluminator border (shown in red).



- 2. When you have positioned the edge guides to your liking, remove the paper tape from under each guide.
- 3. Carefully press each edge guide into position.
- 4. To visualize a sample using the screen, place the screen between the edge guides.
- 5. Use the gel alignment template kit to center the gels on top of the screen consistently. For more information, see To use a gel alignment template with a conversion screen on page 58.

To calibrate the imager

- In Image Lab™ software, choose Edit > Instrument Setup.
- 2. Select the White Light Conversion Screen checkbox under Illumination Options.



3. Under Instrument Calibration, click Reset for Focus Calibration.

When calibration finishes, the imager is ready to acquire images.

To image a gel with the white light conversion screen

- 1. Center the conversion screen on the imager stage.
- 2. Center the samples on top of the conversion screen.
- 3. Image the gel.

For more information, see Chapter 3, Acquiring Images.

XcitaBlue Conversion Screen

The optional XcitaBlue[™] conversion screen kit (catalog #1708182) converts UV to blue light, so you can visualize DNA samples while protecting them against UV damage.



The XcitaBlue conversion screen is held in place by adhesive-backed edge guides. After the edge guides are installed, the conversion screen remains centered and does not slide even when you close the drawer rapidly.

Note: After you install the conversion screen, you must calibrate the imager.

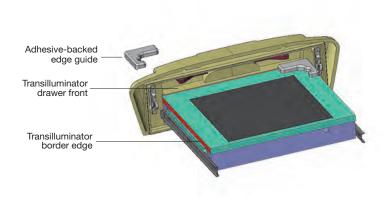
To install the XcitaBlue conversion screen

Important: Do not remove the adhesive paper tape from the edge guides until step 2. You cannot reposition the adhesive surfaces of the edge guides once they are set in place.

1. Holding the screen right side up with the paper taped edge guides underneath, practice positioning the edge guides so that they touch the inside of the

C | Accessories

transilluminator drawer front and fit over the edge of the metal transilluminator border (shown in red).



- 2. Position the edge guides to your liking.
- 3. Remove the paper tape from under each guide.
- 4. Carefully press each edge guide into position.
- To visualize a sample using the screen, place the screen between the edge guides.
- 6. Use the gel alignment template kit to center the gels on top of the screen consistently.

To calibrate the imager

- 1. In Image Lab software, choose Edit > Instrument Setup.
- 2. Under Illumination Options, select the XcitaBlue Conversion Screen checkbox.



3. Under Instrument Calibration, click Reset for Focus Calibration.

When calibration finishes, the instrument is ready to acquire images.

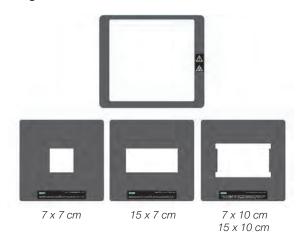
To image a gel on the blue light conversion screen

- 1. Center the conversion screen on the imaging stage.
- 2. Center the samples on top of the conversion screen.
- 3. Select a protocol and image the gel.

For more information, see Chapter 3, Acquiring Images.

Gel Alignment Template Kit

The Bio-Rad gel alignment template kit (catalog #1708184) enables you to center four sizes of standard agarose gels quickly and ensures the consistent placement of each gel.



Note: Using the gel alignment template kit does not affect imager calibration.

The kit contains

- Magnetic locator frame
- Instruction sheet
- Alignment guides for the following gel trays:

- Sub-Cell[®] GT UV-transparent mini-gel tray, 7 x 7 cm
- П Sub-Cell GT UV-transparent wide mini-gel tray, 15 x 7 cm
- Sub-Cell GT UV-transparent mini-gel tray, 7 x 10 cm П
- Sub-Cell GT UV-transparent gel tray, 15 x 10 cm

The gel alignment templates fit exactly into the XcitaBlue conversion screen frame (catalog #1708182).

To use a gel alignment template with a conversion screen

- Remove the magnetic locator frame if it is on the transilluminator.
- Place the conversion screen on the transilluminator.
- 3. Place the gel alignment template that matches the size of the sample tray or agarose gel inside the conversion screen frame.
- 4. Place the gel or gel tray into the open area of the template.

To use a gel alignment template with the magnetic locator frame

- Place the magnetic locator frame over the transilluminator with the magnetic side down.
- 2. Match the corners of the magnetic locator frame with the edges of the transilluminator.
- 3. Position the magnetic locator frame so that its UV symbol matches the direction of the UV symbol on the imager.

For example:





- Place the gel alignment template that matches the size of the sample tray or agarose gel into the magnetic locator frame.
- 5. Place the gel or gel tray into the open area of the template.

Orange Fluorescence Reference Plate

The orange fluorescence reference plate enables you to apply UV flat fielding corrections to the imager. Corrections are made for all UV illumination sources, filters, and the camera lens.

The orange fluorescence reference plate is recommended for quantitation with Bio-Rad's stain-free gels and can correct for image nonuniformities for other fluorescent applications, including the following:

- Ethidium bromide
- GelRed
- Flamingo™
- Coomassie Fluor Orange
- SYPRO Ruby
- Krypton
- Qdot 625



The orange fluorescence reference plate (catalog #1708008) can be used with several of the Bio-Rad® Molecular Imager® series of products, including

- Gel Doc™ XR+ imaging system with Image Lab™ software
- ChemiDoc™ XRS+ imaging system with Image Lab software

Orange fluorescence reference plate, overall dimensions: 29.5 x 29.5 cm; viewing surface: 27 x 27 cm, ships with Instruction sheet (10017296).

Calibrating the Imager for the Orange Fluorescence Reference Plate

You must recalibrate the imager before using the orange fluorescence reference plate.

To recalibrate the imager

- Choose Edit > Instrument Setup.
- 2. In the Edit menu, click Instrument Setup.

The Instrument Setup dialog box appears



3. Under Flat Field, click Reset and then click OK. Follow the instructions that appear on screen.

Ordering Information

The following table lists catalog numbers and descriptions for all parts available for the Gel Doc XR+ and ChemiDoc XRS+ systems, plus all optional accessories and replacement parts. For additional information, see the Bio-Rad catalog.

Table 3. Ordering information

lable of Ordering information				
Catalog #	Description			
Molecular Imager System (includes universal hood, camera, cables, and accessories)				
1708195	Gel Doc XR+ with Image Lab software system for PC/Mac			
1708265	ChemiDoc XRS+ with Image Lab software system for PC/Mac			
Installation Kits				
1708199	Image Lab™ Upgrade for Gel Doc XR+ System			
1708299	Image Lab Upgrade for ChemiDoc XRS+System			
Image Lab Software				
1709690	Image Lab software, Windows/Mac			
Optional Filters				
1708074	Filter, 520DF30, GFP			
1708075	Filter, 560DF50, Cy3/rhodamine			
1708076	Filter, 630BP30, SYPRO Ruby			
Optional Accessories				
1708289	UV/White light conversion screen (UV to white light)			
1708182	XcitaBlue (UV to blue light) conversion screen kit, without standard detection filter			
1708008	Orange fluorescence reference plate			
1703759	Bio-Rad fluorescent ruler			
1703760	Gel cutter ruler			
1708184	Gel alignment template kit			

Table 3. Ordering information, continued

Catalog #	Description			
Replacement Parts				
1708026	Focus calibration target			
1708027	Image Lab flat fielding disc			
1708185	XcitaBlue viewing goggles			
1002784	UV transilluminator lid (includes UV filter glass)			
10014106	Thumbscrew for camera			
1708081	Standard emission filter replacement, 62 mm			
1001370	UV bulb starter, quantity 3			
Lamps				
1002827	Lamp, epi-illumination, 5 W			
1708097	302 nm lamp kit, (6 lamps)			
1706887	365 nm lamp kit, (6 lamps)			
Fuses				
9008935	Fuse T 2 A, 250 V, quantity 10			
1900234	Fuse T 4 A, 250 V, quantity 2			
Optional Analysis Software				
1709600	Quantity One software			
1708205	2.5 kb molecular ruler			
1708200	AmpliSize® molecular ruler			
1708356	EZ Load precision molecular mass ruler (base pairs/ng of sample)			

Using Bio-Rad Stain-Free **Technology**

Bio-Rad stain-free gels eliminate the time-consuming staining and destaining steps required by other protein detection methods. Stain-free gels include unique trihalo compounds that allow rapid fluorescent detection of proteins with the Gel Doc™ XR+ and ChemiDoc™ XRS+ imagerswithout staining.

When using Image Lab™ software, the Gel Doc XR+ and ChemiDoc XRS+ imagers are stain-free enabled to image the following gels:

- Criterion™ TGX Stain-Free™ precast gels
- Criterion Stain Free™ precast gels
- Mini-PROTEAN® TGX Stain-Free™ precast gels
- TGX Stain-Free™ FastCast™ acrylamide solutions for handcast gels

When trihalo compounds in the gels encounter tryptophan residues, a UV light-induced reaction produces fluorescence, which can be easily detected by the imager in gels or on low fluorescence polyvinyl difluoride (PVDF) membranes. Activation of the trihalo compounds in the gels adds 58 Da moieties to available tryptophan residues and is required for protein visualization. Proteins that do not contain tryptophan residues cannot be detected using this technology. The sensitivity of stain-free gels is comparable to staining with Coomassie Brilliant Blue for proteins with a tryptophan content >1.5%; sensitivity superior to Coomassie staining is possible for proteins with a tryptophan content >3%.

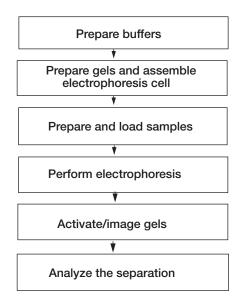
The benefits of stain-free technology include

Elimination of staining and destaining steps for faster time to results

- No background variability within a gel or between gels (as is often seen with standard Coomassie staining)
- Elimination of the need for acetic acid and methanol in staining and destaining, which reduces organic waste
- Visualization of transferred or blotted proteins on low fluorescence PVDF membranes

Stain-Free Workflow

For detailed information about the Activate/image gels step, refer to Chapter 3, Acquiring Images. For all other workflow steps, refer to the Criterion™ Precast Gels Instruction Manual and Application Guide (bulletin #4110001) or to the Mini-PROTEAN® Precast Gels Instruction Manual and Application Guide (bulletin #1658100).



Electrophoresis with Stain-Free Gels

Stain-free gels are made and packaged without sodium dodecyl sulfate (SDS), allowing them to be used for both SDS and native polyacrylamide gel electrophoresis (PAGE) applications.

To perform electrophoresis with stain-free gels

- Prepare the sample and running buffers.
- 2. Set up the electrophoresis cell.
- Perform the run.

Imaging Gels

Use unstained standards with stain-free gels, as some prestained standards are not compatible with stain-free technology. To monitor electrophoresis, use a 1:1 mixture of unstained and prestained standards.

Setting up a protocol for stain-free gels is similar to setting up protocols for other applications. Follow the instructions in Creating a Protocol on page 20. Choose one of the following activation times based on the sample and the purpose of your experiment:

- **Gels used in blotting** use 1 min activation for optimal results when performing western blotting followed by immunodetection.
- Good sensitivity use 2.5 min activation when samples are abundant and when a fully optimized signal-to-noise ratio is not necessary.
- Best sensitivity use 5.0 min activation for detection of proteins that are in low concentration and for the best quantitation of the maximum number of bands. Because the reaction is near completion after 5 min, this method offers an optimal signal-to-noise ratio.

Note: If the gel has been activated for 2.5 min, activating it for another 2.5 min might improve it. But activating an image for more than 5 min will not.

Imaging Blots

To blot stain-free gels, use standard blotting procedures as described in the instruction manual you are using. Use only PVDF membranes with low background fluorescence, as membranes other than low fluorescence PVDF can result in high background or low sensitivity with the imager.

To assess transfer efficiency, be sure to activate and visualize the gel using the imager before transfer.

Glossary

Aspect ratio	The ratio of the width to the height of an image.		
CCD	(Charge-coupled device) A light-sensitive silicon chip used as a photodetector in Gel Doc™ XR+ and ChemiDoc™ XRS+ camera systems.		
Colormaps	Different color representations of a gel image.		
Electrophoresis	A technique for separating molecules based on the differential movement of charged particles through a matrix when subjected to an electric field.		
Example precision	The number of decimal places chosen for displaying a measurement.		
Flat fielding	An average intensity computation that compensates for nonuniformities generated by an instrument.		
Histogram	A graphed representation of the brightness, or gray value, of an image.		
Imager	The instrument without Image Lab software.		
Imaging system	The instrument connected to a computer running Image Lab™ software.		
Native charge density	The inherent electrical charge of a protein without the addition of SDS.		
pl	Isoelectric point; the pH at which a protein molecule carries no net charge.		
Quantitative imaging	Determines the quantity of a protein's components through analysis of the pixel values in a digital image of the sample.		
UV-B	The range of ultraviolet light used by the system.		
UV transilluminator	The part of the imager that transmits UV light through a sample.		

Glossary



Bio-Rad Laboratories, Inc.

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